Application Note for ViroMag RL mediated viral vectors-based Genome Edition using the CRISPR-Cas9 system

IMPORTANT NOTES – Before you begin

- ✓ ViroMag RL must be stored at 4°C and used according to the table 1. Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ Order of addition is important: add the viral solution onto ViroMag RL magnetic nanoparticles.
- ✓ Dilute ViroMag RL with deionized water for doses less than 1µL.
- ✓ **Medium or buffer without serum & supplement** must be used for the preparation of the complexes. Culture medium such as DMEM or OptiMEM or buffers such as HBS or PBS are recommended. Alternatively, you can directly use an aliquot of the culture supernatant from a producer cell line.
- ✓ For cell in suspension, seed the cells just before starting transduction experiment; you can draw inspiration from Sacha JB et al. Nat Protoc. 2010 working on T lymphocytes infection with ViroMag RL.
- ✓ Polybrene or other additives must NOT be used in combination with ViroMag RL
- ✓ **The suggested volume of ViroMag RL is related to infectious particles and not physical viral particles.** Adapt the MOI depending of the viral vector and the type of cells used. MOI can usually vary from 0.5 up to 100 and adjust ViroMag RL volumes accordingly.
- ✓ For sensitive cells, medium can be replaced with fresh complete culture medium 4 to 6h after transfection or right after the magnetofection procedure.
- ✓ Do not freeze the magnetic nanoparticles!

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

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Any questions?

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1. Cells preparation

It is recommended to seed or plate the cells the day prior transfection. The suitable cell density will depend on the growth rate and the cells conditions. Cells should be 60-90% confluent at the time of transduction (refer to table 1).

Tissue Culture Dish	Adherent Cell Number	ViroMag RL (µL)	Recommanded ViroMag RL (µL)	Volume for complexes (µL)	Transfection volume
96 well	0.5 – 2 x 10^4	0.2 – 3	1.5	50	200µL
24 well	0.5 – 1 x 10^5	1 - 12	6	100	500µL
6 well	2 – 4 x 10^5	5 - 60	30	200	2mL

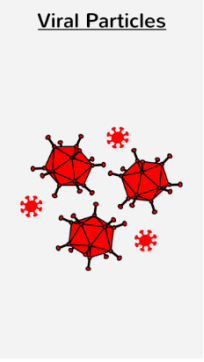
Table 1: Suggested transfection conditions

2. Viral solution preparation

Effective gene-editing using the CRISPR-Cas9 system can only be achieved through efficient delivery of all elements into target cells. […] generally, all-in-one lentiviral vectors expressing both Cas9 and sgRNAs were designed and constructed (Shalem O., et al. *Cells. Science*. 2014).

Cas 9 and guide RNA (gRNA) are generally thus encoded by a single viral vector or two different ones.

Prepare a viral particle solution composed by either one viral vector or vectors encoding cas9 and sgRNA.



Viral vectors encoding CRISPR reagents

3. Viral particles/ViroMag RL complexes preparation

a. *ViroMag RL*: Vortex the reagent and place the appropriate amounts (refer to table 1) in an empty microtube.

b. *Viral particles solution*: Add your virus suspension to the tube containing ViroMag RL and mix immediately by pipetting up & down. Do not vortex

Note: Prefer virus solutions made in serum-free medium or salt-containing buffers.

c. Incubate at room temperature for 15 to 20 minutes.

4. Transduction

a. Add the ViroMag RL / virus complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution. Place the cell culture plate on the magnetic plate during 30 minutes.

b. Remove the magnetic plate.

c. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression (from 24h up to 7 days).

NOTES:

- In case of cells very sensitive to transfection, the medium can be changed right after the Magnetofection procedure
  - ➔ keep cells onto the magnetic plate and replace the transfection medium with fresh pre-warmed complete culture medium.
- Some cell types need medium change 2 - 4h after transfection.

Notes for suspension cells

d. Prepare complexes of viral particles and ViroMag RL as recommended above.
e. After incubation, add complexes to the cell in suspension
f. Centrifuge the cells and the complexes 5 min at 150xg
g. Remove carefully the plate from the centrifuge and place it onto the magnetic plate for Magnetofection procedure
h. Incubate 30 min, remove the magnetic plate and cultivate the cells under standard culture conditions until evaluation of the experiment.

Note: you can refer to the following papers for a detailed protocol for transduction and synchronous infection of suspension cell (T lymphocyte with ViroMag RL) :

- *Sacha JB et al. Nat Protoc. 2010 Feb;5(2):239-46. doi: 10.1038/nprot.2009.227*
- *Barsov EV. Methods Mol Biol. 2009;511:143-58. doi: 10.1007/978-1-59745-447-6_6.*

Optimization Protocol

Several parameters can be optimized:

- ViroMag RL dose & Ratio to viral particles
- cell density and incubation times

1) Start by optimizing the ViroMag RL dose with a fixed MOI. This will vary the concentration of ViroMag RL and the ratio ViroMag RL / Virus. To this end, vary the amount of ViroMag RL in the range suggested in the Table 1. For instance, from 0.2 to 3µL of ViroMag in a 96-well plate.

2) Next, use a fixed volume of ViroMag RL and vary the MOI.

3) Finally, you can optimize the cell number (density), kinetics of readout and also the incubation time for the magnetofection procedure.

Additional transfection reagents for CRISPR/Cas9 Genome Editing

- **Pro-deliverIN CRISPR** for Cas9 protein delivery
- **RmesFect** for Cas9 mRNA transfection
- **PolyMag Neo** for the co-transfection of plasmids encoding Cas9 and guide RNA

Purchaser Notification
Limited License
The purchase of the ViroMag RL kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the ViroMag RL kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all ViroMag RL kit reagents and documentation to OZ Biosciences, or by destroying all ViroMag RL components. Purchasers are advised to contact OZ Biosciences with the notification that a ViroMag RL kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the ViroMag RL kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.
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